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ACCURACY ASSESSMENT OF *Syringa vulgaris* L. MORPHOLOGICAL PHENOTYPING WITH A LASER 3D SCANNER PlantEye F500 DEPENDING ON PLANT LOCATION ON THE SCANNED SURFACE

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Abstract

Since the methodological methods of direct genetics are applicable only for monogenic traits, the created breeding material, line or variety must be tested in the field, since the presence of the desired gene in the genome, confirmed by molecular methods, does not always lead to the formation of a trait valuable for selection. Systems based on 3D imaging technologies make it possible to obtain a plant model, as well as information on morphological parameters. However, very little attention is paid to the preparation of protocols for phenoscreening. The purpose of this study was a comparative assessment of the accuracy of determining the morphological characteristics of lilac plants by traditional methods and using machine vision technology, depending on the plant location on the scanned surface. Microclones of lilac (*Syringa vulgaris* L.) cv. Microclones are morphologically homogenous and small in size, which allows measurements of sufficiently large sets of samples and makes it easier to compare the research results by their normalization to average values. The measurements were made after the plant complete adaptation and cultivation for 1 month in a greenhouse. With traditional morphometry, in 10 microclones, the height was measured with a measuring ruler, and the leaf area was measured using the contour method. When scanning (PlantEye F500 3D scanner, Phenospex B.V., Netherlands), each of 10 selected plants was placed at five different positions of the scanned surface, and at least five repeated scans were performed in the same position. When using machine vision technology, 3D leaf area, projected leaf area, digital biomass, height, maximum height, leaf tilt, leaf tilt angle, light penetration depth were determined. It has been established that in order to obtain objective and comparable data from using a 3D scanner, it is optimal to place plants in the center of the scanned surface in the same position. The following parameters can be recommended to identify varieties and assess plant growth rate: the leaf area, projected leaf area, height, and leaf inclination angle. For each plant species, it is necessary to preliminarily study particular morphological traits and to compare the obtained data with the scan results in order to introduce correction factors/ This will confirm the information content of the feature set used, thereby increasing the accuracy of machine vision technology data.

Keywords: phenotyping, morphology, *Syringa vulgaris* L., machine vision technology, 3D canning

Modern genetic research is focused on the genome structure [1, 2] in order to identify determinants for economically valuable traits and mechanisms of gene acttivity [3-5], to assess population variability [6], to identify varieties at the early stages of plant development [7, 8], to reveal patterns of genome organization and evolution [9, 10].

The widely used approaches of direct classical genetics in which genes are

identified by the traits they encode are being replaced by reverse genetics methods when not the phenotype and its genetic control are analyzed but the DNA sequence itself and its phenotypic effects are revealed [11-14]. The paradigm shift is due to the fact that the methodological methods of direct genetics are applicable only for monogenic traits. However, in most cases, the properties of biological objects are polygenic and are formed as a result of the combined action of several genes, or phenotypic expression may be the result of mutations in different genes [15]. Therefore, the traits of the obtained breeding material, lines or varieties should be checked in the field, since the presence of the desired gene in the genome confirmed by molecular methods does not always lead to the formation of a trait valuable for breeding [16-18]. In addition, when analyzing qualitative and quantitative morphological features, it is necessary to recognize the modification variability that occurred due to various environmental factors [19].

The morphological characterization of plants is an obligatory stage of selection and genetic studies [15, 17]. Modern phenotyping methods based on machine vision technologies are highly productive and allow obtaining real-time data on several morphological parameters [20, 21]. Automation of phenotyping processes significantly speeds up the analysis and increases its accuracy, eliminating the human factor as a source of subjective evaluation of the results, and provides with parameters that were not used in traditional morphometric measurements [22].

The most important morphological features in plant phenotyping include plant size, type of leaf arrangement, shape and area of the leaf blade. There are automated platforms that allow identification of plant species from photographs, such as INaturalist (https://www.inaturalist.org/) and PlantNet (https://plantnet.org/). However, the accuracy of phenotyping depends on the accumulated photographic material (the number and quality of photographs at different stages of plant vegetation), the frequency of occurrence of the species in the study area, and the actual confirmation of its identification during field observations [23, 24]. Thus, when using automated platforms, it is possible to determine plant species with sufficient content of the database, but it is not possible to assess the modification variability of morphological characters, as well as to determine varieties.

Systems based on 3D imaging technologies provide a plant model as well as information on morphological parameters [25]. In this case, the image processing software plays a major role, and not the resolution of the scanner [26]. As a result, current research on phenotyping is mainly devoted to software development, improvement of the camera positioning system [27]. However, very little attention has been paid to the development of protocols for phenoscreening [28-30]. There is no doubt that the automation of phenotyping processes, carried out both in laboratory and in the field, will not only significantly speed up the evaluation of breeding material, but will also increase the homogeneity of selected plants when working with annual crops [31-34]. Despite many publications on the use of 3D scanners for assessing morphological parameters, the literature covers rather superficially the issues of accuracy of morphological characteristics in plant phenotyping depending on their location on the scanned surface [35-37].

In this paper, we compared the results of direct morphometric measurements carried out by personnel and indirect measurements based on machine vision technology, and identified conditions that, if not observed during phenoscreening, can lead to unreliable results.

The purpose of our study was to comparatively assess the accuracy of determining morphological traits of lilac plants by traditional methods and by machine vision technology, depending on the location of the object on the scanned

surface.

Materials and methods. Lilac plants (*Syringa vulgaris* L.) cv. Mercy were obtained by the in vitro method after adaptation. Accounts were made after the completion of the stage of adaptation and cultivation of plants for 1 month in greenhouses.

With traditional morphometry, the sample consisted of 10 microclones, in which plant height was measured with a ruler, and the surface area of each leaf was measured by the contour method.

Scanning was performed on a PlantEye F500 multispectral 3D unit (Phenospex B.V., the Netherlands) (equipment of UNU Botanical Garden of Belgorod State National Research University, https://ckp-rf.ru/usu/200997/). Each of the 10 selected plants was scanned at five different points on the scanned surface, and at least five repeated scans were performed in the same position. Using the PlantEye F500 setup, the values of the following morphometric parameters were analyzed: 3D Leaf Area, cm^2; Projected Leaf Area, cm^2; Digital Biomass, cm^3; Height, mm; Height Max, mm; Leaf Inclination, cm^2/cm^2; Leaf Angle, °; Light Penetration Depth, mm. For processing the obtained data, the PlantEye F500 HortControl software was used.

Arithmetic mean values (M) and confidence intervals (±CI) were calculated at a confidence level $p = 0.05$, and correlation analysis was performed.

Results. The choice of microclones as an object is due to a high degree of morphological uniformity and small plant sizes, which allows measurements and comparison of the data obtained in sufficiently large samples, normalizing them to average values.

At the first stage of the study, we carried out morphometric measurements of plant height (22.7±2.3 cm) and leaf surface area (388.3±12.3 cm^2). Digital biomass (product of plant height and leaf surface area) was 8814.41±325 cm^3.

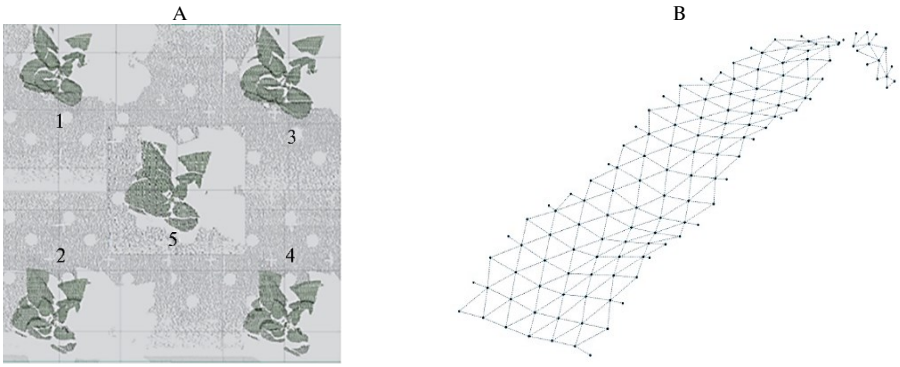

Fig. 1. Positioning of lilac (*Syringa vulgaris* L.) cv. Mercy plants (A) **and triangulation of points to generate a 3D cloud** (B) **for phenotyping by 3D scanning** (PlantEye F500, Phenospex B.V., the Netherlands).

The position of each plant during 3D scanning is shown in Figure 1, A. When using the PlantEye F500 3D scanner to measure leaf area, points are created in the point cloud that belong to the same array, which are triangulated (connected into triangles). Since an uneven distribution of points in space is allowed, the size of the triangles can vary (see Fig. 1, B).

A group of triangles forming a uniform surface represents a domain and corresponds to one sheet. Then the total area of 3D scanning of plant leaves is calculated as the sum of the areas of elementary triangles of all scanned leaves of one plant (Fig. 2).

The presented data show that plant locations significantly affected the result. At points 2 and 4, the smallest values of the leaf area were obtained, at points

3 and 5, the largest, and the location of the plant at point 1 corresponded to the average value obtained at all five points. It should also be noted that the confidence interval for five repeated scans in 10 plants turned out to be the largest at point 1.

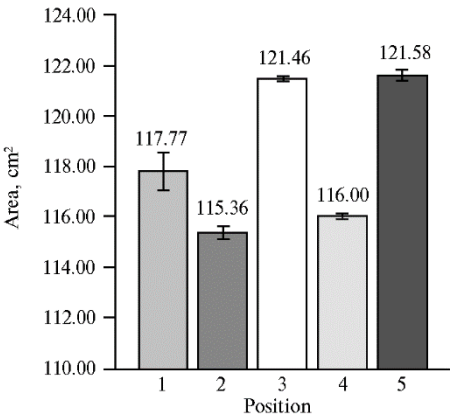

Fig. 2. Leaf area depending on posiooning of of lilac (*Syringa vulgaris* L.) cv. Mercy plants (n = 10, M±CI, p = 0.05; 3D scanning, PlantEye F500, Phenospex B.V., the Netherlands). Each plant was placed at 5 points, and at least 5 repeated scans were performed in the same position.

That is, when conducting automated measurements, the data obtained are affected by the position of the plant relative to the scanning area. Even with a static location of the object and the absence of external changing factors (changing the illumination did not affect the scan results) at the scan point closest to the beginning of the scan area, a high instability of the obtained data occurred.

The total leaf area per plant when using the contour method was 3.2 times higher than that in 3D scanning. This significant discrepancy is due to the fact that some leaves overlap each other, which underestimates the figure. Therefore, the use of a 3D scanner to estimate leaf area requires the introduction of a correction factor calculated on the basis of a comparison of data obtained by different methods (in our case, the contour method and as a result of scanning with the PlantEye F500).

The projected leaf area is defined as the projection area of all elementary triangles onto the X-Y plane. However, it is equivalent to a value that can be measured with a conventional 2D camera. PlantEye F500 measures the projection area of the plant on the X-Y plane and turns the 3D object into a flat 2D object (Fig. 3).

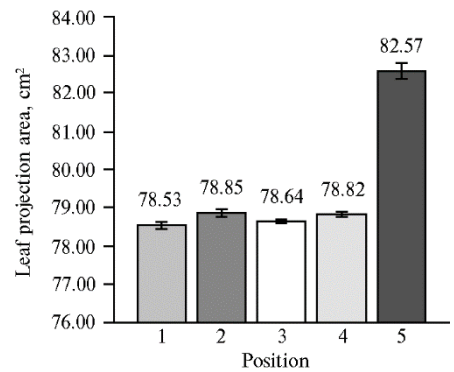

Fig. 3. Leaf projection area depending on posiooning of of lilac (*Syringa vulgaris* L.) cv. Mercy plants (n = 10, M±CI, p = 0.05; 3D scanning, PlantEye F500, Phenospex B.V., the Netherlands). Each plant was placed at 5 points, and at least 5 repeated scans were performed in the same position.

It can be seen from the histogram that the location of the plant at points 1, 2, 3 and 4 did not significantly affect the obtained data, while the location in the center of the scanned surface (point 5) led to both a significant increase in the leaf area in the projection and an increase in the confidence interval. The average values of the area of all leaves for 10 plants and the projected area of the leaves obtained by 3D scanning are correlated (r = 0.55, p < 0.05). At the same time, the value of the projected leaf area is much less than the leaf area, since in 3D scanning, leaves with the same position in the X-Y plane, but located at different heights above the ground, are not counted twice. That is, the projected leaf area serves as an analogue of the projective cover, which determines the relative leaf projection area on the underlying surface.

With the PlantEye F500 instrument, digital biomass is calculated as the

product of height and leaf area values, provided that the plant has a shoot structure, the volume of which can be calculated from height and length (Fig. 4).

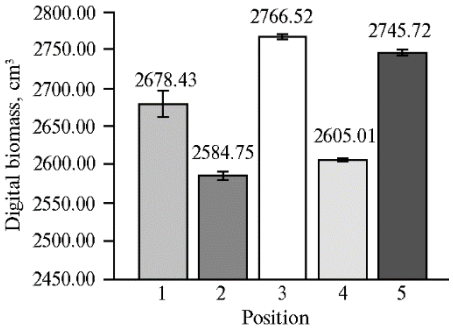

Fig. 4. Digital leaf biomass depending on positioning of of lilac (*Syringa vulgaris* L.) cv. Mercy plants (n = 10, M±CI, p = 0.05; 3D scanning, PlantEye F500, Phenospex B.V., the Netherlands). Each plant was placed at 5 points, and at least 5 repeated scans were performed in the same position.

Similarly, the location of the plant influenced the r assessment of both digital biomass and leaf area (r = 0.98, p < 0.05). Points 2 and 4 showed the smallest digital biomass, points 3 and 5 showed the largest one, and point 1 corresponded to the average value from all five points, at point 1 the value of the confidence interval was also the largest, as in the case of leaf area (see Fig. 2).

To calculate plant height, PlantEye F500 uses the distribution of elementary triangles along the Z axis. To do this, a histogram along the Z axis is first calculated, which reflects the number of elementary triangles at different heights above the ground. Next, the top 10% of the plant height is averaged, and the height itself is calculated as the distance from the height of the pot to the part for which the averaging was performed (Fig. 5).

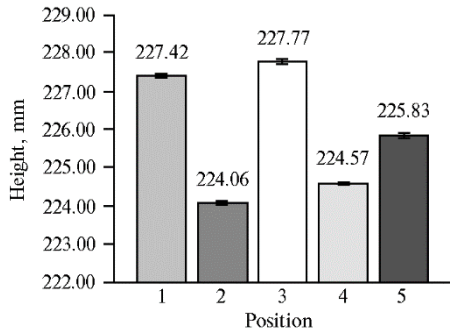

Fig. 5. Plant height depending on positioning of lilac (*Syringa vulgaris* L.) cv. Mercy plants (n = 10, M±CI, p = 0.05; 3D scanning, PlantEye F500, Phenospex B.V., the Netherlands). Each plant was placed at 5 points, and at least 5 repeated scans were performed in the same position.

It can be seen from the histogram that the location of the plant at points 1 and 3 gave the maximum height values, at points 2 and 4 the minimum, while the location of the plant in the center of the scanned surface (point 5) corresponded to the average value for all five points. It should be borne in mind that the error in the obtained values is determined by how deep the plant is located relative to the edge of the pot, and can be from 1 to 5 cm.

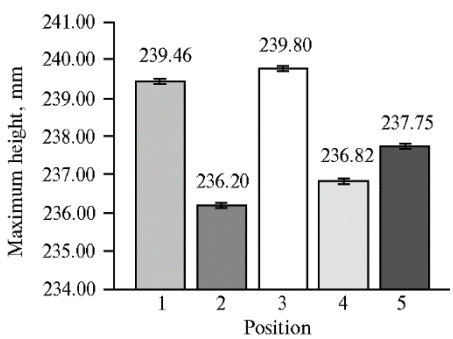

Fig. 6. Maximun plant height depending on positioning of lilac (*Syringa vulgaris* L.) cv. Mercy plants (n = 10, M±CI, p = 0.05; 3D scanning, PlantEye F500, Phenospex B.V., the Netherlands). Each plant was placed at 5 points, and at least 5 repeated scans were performed in the same position.

The maximum height is designed to define the absolute highest point of the plant in millimeters. This indicator does not replace the current height setting, but complements it. The current height focuses on averages rather than measurement accuracy, minimizing the effect of external artifacts or daily plant movements. To calculate the maximum height, PlantEye F500 finds the highest area (a group of

points in a 3D file) that contains the required number of points and is close enough to other areas. In this domain, the highest point is then given as the maximum height (Fig. 6).

In general, for the height and maximum height of plants during 3D scanning, the same dependence of the change in indicators for different positions of the plant on the scanned surface can be traced (for the correlation between these two parameters, $r = 0.99$, $p < 0.05$). The difference between the height and maximum height values in our experiment was about 12 mm at all points.

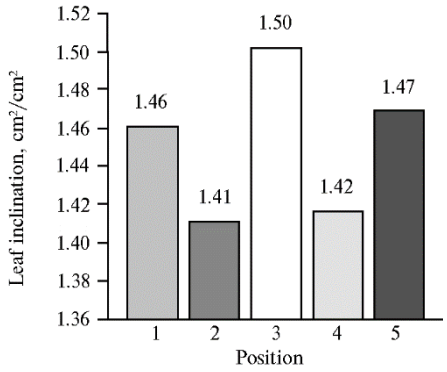

Fig. 7. Leaf inclination depending on positioning of lilac (*Syringa vulgaris* L.) cv. Mercy plants ($n = 10$, $M \pm CI$, $p = 0.05$; 3D scanning, PlantEye F500, Phenospex B.V., the Netherlands). Each plant was placed at 5 points, and at least 5 repeated scans were performed in the same position.

The leaf slope reflects information about how high the leaves are on the plant and is calculated as the total leaf area divided by the sum of the projections of each elementary triangle onto the X-Y plane (Fig. 7). The confidence interval for the obtained values is so small that it can be neglected with five repeated measurements in 10 plants. The maximum leaf slope values were obtained at point 3, the minimum values were obtained at points 2 and 4.

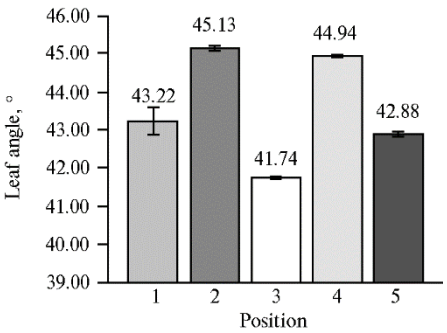

Fig. 8. Leaf angle depending on positioning of lilac (*Syringa vulgaris* L.) cv. Mercy plants ($n = 10$, $M \pm CI$, $p = 0.05$; 3D scanning, PlantEye F500, Phenospex B.V., the Netherlands). Each plant was placed at 5 points, and at least 5 repeated scans were performed in the same position.

The leaf angle is the arithmetic mean of all the angles of each facet based on their normal (Fig. 8).

The presented histogram shows that the location of the plant on the scanned surface significantly affects the obtained values. Thus, the location at points 2 and 4 gives the maximum values of the leaf angles, at point 3 the minimum, at points 1 and 5 the values are closest to the average for all five points. The slope angle was inversely proportional to the slope of the leaves ($r = -0.99$, $p < 0.05$) and leaf area ($r = -0.92$, $p < 0.05$) in 3D scanning. The higher the leaves are above the ground and more rotated relative to the scanning element, the greater the total leaf area will be (without changing the predicted area), and as a result, the slope of the leaves will increase.

The depth of penetration of light reflects the distance that the laser beam can penetrate through the leaf surface of the plant (Fig. 9). From the presented data, it can be seen that the location of plants at points 1, 2 and 3 did not significantly affect the depth of light penetration, at point 4 there was a slight decrease in the indicator, and at point 5 we noted the lowest degree of penetration of the laser beam. Thus, we can state with confidence that the location at point 5 is the most informative. The value of the projected leaf area in different locations of the plant on the scanned surface is inversely proportional to the depth of light

penetration (r = –0.95, p < 0.05).

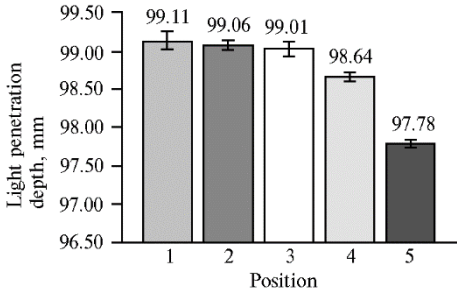

Fig. 9. Light penetration depth depending on positioning of lilac (*Syringa vulgaris* L.) cv. Mercy plants (n = 10, M±CI, p = 0.05; 3D scanning, PlantEye F500, Phenospex B.V., the Netherlands). Each plant was placed at 5 points, and at least 5 repeated scans were performed in the same position..

Thus, our study showed that the location of the plant on the scanned surface significantly affects the values of morphological parameters measured using the PlantEye F500 3D laser scanner. Of the eight morphological parameters, two (plant height and maximum plant height) duplicate each other. When analyzing plant size, plant height is more preferable, since the confidence interval was smaller with five height measurements. The value of leaf area and digital biomass at different points of plant location on the scanned surface correlate (r = 0.98). Digital biomass is a less informative indicator for 3D scanning. It has a large confidence interval and depends on the plant architectonics. A necessary condition for determining this parameter is the ability to calculate the volume given the height and the plant length. Two indicators with an inverse relationship (r = –0.95) are the leaf projected area and the depth of penetration of the laser beam. The depth of penetration of light could be an interesting indicator of the density of shoots and leaves. Howevere, with small plants, as it was in our study since we used plants grown in vitro, these parameters are not significant. The value of the angle of inclination is inversely proportional to the inclination of the leaves (r = –0.99). However, the leaf tilt angle is a more informative indicator in 3D scanning, allowing a better understanding of the architectonics of the plant, despite the fact that the confidence interval for leaf tilt is almost zero. In any case, both of these values are calculated based on the average of all leaf slopes and slope angles, therefore, a decrease in shoot turgor can significantly affect the results obtained.

The analysis of publications and the results obtained by us of the practical application of machine vision technologies in assessing the morphological parameters of lilac plants of the Mercy variety using the PlantEye F500 3D scanner made it possible to identify the following advantages and disadvantages of the automated approach. The advantages include the fact that phenotyping platforms allow one scan to determine from 5 to 15 morphological characteristics on one or several plants at once [38-40]. Carrying out measurements of morphological parameters by traditional methods requires the use of various types of equipment, as well as significant labor costs. The accuracy of the obtained values of various morphological parameters is characterized by a high degree of convergence (see Fig. 2-9) even despite the existing measurement errors. The obtained data are loaded into a computer, and it is possible to assess the dynamics of changes in morphological parameters over time. The lack of protocols for phenoscreening of morphological parameters for different crops should be considered as a disadvantage [41]. It should be taken into account that when several plants are studied simultaneously on the scanned surface, the probability of measurement error increases, as indicated by our experimental data. The impossibility of using the installation in the field during experiments, external factors (wind) prevent the plant from remaining in a static position and, as a result, affect the accuracy of the data obtained [36]. Most of the morphometric parameters studied by 3D scanning are in strong positive or negative interdependence and duplicate each other, for example, height and maximum height, digital biomass and leaf area, projected leaf

area and light penetration depth. That is, software developers need to focus not on the number of output parameters, but on their informativeness in assessing the state of plants and the possibility of determining the dynamics of growth processes. when using various forms of drugs. Based on the fact that when changing the position of the same plant on the scanned surface (moving along five points), the recorded morphological parameters differed significantly, it can be confidently expected that the location of several plants on the scanned surface will lead to significant differences in the data obtained.

Thus, our finndings show that, when using the PlantEye F500 3D scanner, it is optimal to place plants in the center of the scanned surface in the same position in order to obtain objective and comparable results. As morphological parameters for identifying varieties and fixing growth, we can recommend using the leaf area parameters, projected leaf area, plant height, and leaf angle. For each plant species, it is necessary to conduct primary morphological studies using traditional methods, and then compare the obtained data with the scan results to calculate the correction factor and confirm the information content of the trait set used, thereby increasing the accuracy of the data provided by machine vision technology.

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